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Evaluating Potential Response-Modifying Factors for Associations between Ozone and Health Outcomes: A Weight-of-Evidence Approach

Lisa C. Vinikoor-Imler,[1] Elizabeth O. Owens,[1] Jennifer L. Nichols,[2] Mary Ross,[1] James S. Brown,[1] and Jason D. Sacks[1]

[1]National Center for Environmental Assessment (NCEA), U.S. Environmental Protection Agency (EPA), Research Triangle Park, North Carolina, USA; [2]Oak Ridge Institute for Science and Education, at National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA

Address correspondence to Lisa C. Vinikoor-Imler, 109 T.W. Alexander Drive, Attn: MD B243-01, Research Triangle Park, NC 27711 USA. Telephone: 919-541-2931. E-mail address: vinikoor-imler.lisa@epa.gov

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Abstract

Background: Epidemiologic and experimental studies have demonstrated a variety of health effects in response to ozone (O_3) exposure. Studies have demonstrated that some populations may be at increased or decreased risk of O_3-related health effects.

Objectives: To identify potential response-modifying factors to determine if specific groups of the population or lifestages are at increased or decreased risk of O_3-related health effects using a weight-of-evidence approach.

Methods: Epidemiologic, experimental, and exposure science studies of potential factors that may modify the relationship between O_3 and health effects were identified in U.S. EPA's 2013 Integrated Science Assessment for Ozone and Related Photochemical Oxidants. Scientific evidence from studies that examined factors that may influence risk were integrated across disciplines to evaluate consistency, coherence, and biological plausibility of effects. The factors identified were then classified using a weight-of-evidence approach to conclude whether a specific factor modifies the response of a population or lifestage resulting in increased or decreased risk of O_3-related health effects.

Discussion: We found "adequate" evidence that populations with certain genotypes, preexisting asthma, and reduced intake of certain nutrients, along with different lifestages and outdoor workers, are at increased risk of O_3-related health effects. Additionally, we identified other factors (i.e., sex, SES, and obesity) for which there was "suggestive" evidence that they may increase the risk of O_3-related health effects.

Conclusions: Using a weight-of-evidence approach we identified a diverse group of factors that should be considered when characterizing the overall risk of health effects associated with exposures to ambient O_3.

Introduction

As discussed in the Amendments to the Clean Air Act, the health-based, or primary, National Ambient Air Quality Standards (NAAQS) for the criteria air pollutants (U.S. EPA 2011b), which includes ozone (O_3), are intended to provide an adequate margin of safety that is requisite to protect public health from ambient air pollution, taking into consideration "measures which may be employed to… protect the health of sensitive or susceptible individuals or groups" (Clean Air Act Amendments, 1990). Therefore, as part of the NAAQS process, it is important to thoroughly evaluate the available scientific evidence to accurately identify those populations or lifestages at increased risk of an air pollutant-related health effect. The most recent review of the scientific evidence that supports the NAAQS for O_3 included an evaluation of factors that may increase or decrease the risk of air pollutant-related health effects (U.S. EPA 2013).

It is often recognized that populations can experience increased risk for air pollutant-related health effects at a given concentration as a result of multiple avenues, specifically (1) intrinsic factors, (2) extrinsic factors, and/or (3) increased dose (Samet 2011). Intrinsic factor(s) are often defined as individual characteristics that may increase risk through a biological mechanism (e.g., age, sex, genetics, etc.); whereas, extrinsic factors represent external, non-biological factors, such as socioeconomic status (SES) or access to health care. Some portions of the population may be at increased risk of an air pollutant-related health effect due increased internal dose at a given exposure concentration. Additionally, populations may be at increased risk of an air pollutant-induced health effect due to differential exposure, for example, as a result of being subjected to higher concentrations of an air pollutant through occupations requiring outdoor work, residential locations near areas of higher concentration, or the lack of household air conditioning units to reduce indoor O_3 concentrations (Samet 2011). Factors that modify risk of air pollutant-related

health effects may be multifaceted, resulting in a population being at increased or decreased risk due to more than one of these components.

Identifying populations at increased or decreased risk of an air pollutant-related health effect requires defining the attributes of a population that could render them at increased or decreased risk. Previous reviews, such as Sacks et al. (2011) have introduced the idea of using an all-encompassing term, such as "susceptible" to shift the emphasis away from classifying factors that modify risk into groups such as "susceptible" or "vulnerable", as those terms have been used inconsistently across the literature. This approach, therefore, allows for the focus of any evaluation to be on the fundamental question; *What populations are at greatest risk and what evidence forms the basis of this conclusion?* instead of the categorization of factors. Over time, it has become evident that even the term "susceptible" has underlying connotations and is not accurately capturing the entirety of the factors that could modify the risk of an air pollutant-related health effect. As such, we introduce the term "response-modifying factors" (RMFs), which we define as: any condition or state that changes exposure or response from an environmental pollutant. RMFs can include intrinsic factors, extrinsic factors, factors that result in differences in dose, and/or factors that result in differential exposure.

The focus on the term "response", characterizes the fact that the studies evaluated to determine the factors that increase or decrease risk are not all epidemiologic studies focusing on a change in the relative risk associated with a health effect, but also include experimental studies which focus on understanding if biological responses are changing as a result of air pollutant exposures.

Using the approach originally detailed in Sacks et al. (2011) to integrate evidence across the scientific disciplines and determine whether specific populations or lifestages are RMFs, we

define, evaluate, and characterize the factors that potentially modify the risk of O_3-related health effects, regardless of whether the increased or decreased risk is due to intrinsic factors, extrinsic factors, increased dose, differential exposure, or a combination of these. In order to capture the breadth of information on various potential RMFs, overviews of each factor, as opposed to detailed reviews, are presented. A weight-of-evidence approach is then used to draw conclusions with regard to the level of confidence that a specific factor increases or decreases the risk of an O_3-related health effect. By applying a weight-of-evidence approach, we identify which factors have the strongest evidence to support their status as RMFs. These conclusions can then be used as the scientific basis for future policy decisions intending to protect those portions of the population at greatest risk, as mandated by the Clean Air Act Amendments of 1990.

Methods

Literature search

To evaluate RMFs that may result in a population or lifestage being at increased or decreased risk of an O_3-related health effect, we focus on the collective evidence evaluated in the most recent scientific review of the O_3 NAAQS as presented in the 2013 Integrated Science Assessment (ISA) for O_3 and Related Photochemical Oxidants (U.S. EPA 2013). The O_3 ISA builds upon the conclusions of previous O_3 assessments (e.g., U.S. EPA 2006b, U.S. EPA 1996) and consists of a comprehensive review of papers published in the peer-reviewed literature through July 2012, with the bulk of the studies published from June 2009 through July 2012, that focused on health effects of short-term (i.e., less than one month) and long-term (i.e., months to years) O_3 exposure. These scientific studies were originally identified using an exhaustive literature search strategy in Pubmed and Wed of Science using the terms “ozone”, “O_3”, “smog”, and “photochemical oxidant(s)” (retrieving ~22,000 references). The broad pollutant-based

search was supplemented by various targeted search strategies for the identification of studies that examined specific health endpoints. These more targeted searches were determined based on prior knowledge of the key health endpoints related to O_3 exposure (i.e. respiratory morbidity, cardiovascular morbidity, mortality, reproductive and developmental effects, and cancer) as well as emerging effects in air pollution literature. A detailed explanation of this literature search strategy can be found in the Supplemental Material (Supplemental Material, Literature Search Strategy).

Overall study selection and evaluation of study quality

Once the entire body of scientific literature that examined the effect of O_3 exposure on various health effects was identified, a detailed study selection process was followed by the EPA to identify those studies most relevant (i.e., policy relevant) to the O_3 NAAQS review and evaluate their overall quality (Supplemental Material, Study Selection and Evaluation of Individual Study Quality). Policy-relevant and informative studies include those that provide a basis for or describe the relationship between O_3 exposure and effects, including studies that offer innovative methods or design and studies that reduce uncertainty on critical issues. Emphasis was placed on studies that examine effects associated with O_3 concentrations relevant to current population exposures, and particularly those pertaining to O_3 concentrations currently found in ambient air. However, studies with higher concentrations were included if they contained unique data, such as a previously unreported biological effect or MOA or if they examined multiple O_3 concentrations to elucidate exposure-response relationships. After selecting studies for inclusion, the individual study quality was evaluated by considering the design, methods, and documentation of each study, but not whether the results are positive, negative, or null. This systematic approach to evaluating the literature has been utilized during the reviews of ISAs for

all of the criteria pollutants, including O_3, which undergo extensive review by an independent panel of subject matter experts, the Clean Air Scientific Advisory Committee (CASAC).

Selection of RMF studies

Within the large body of policy relevant studies (~2,200 studies) that examined the relationship between O_3 exposure and health effects and were included in the 2013 O_3 ISA, for this overview, we focused on a subset of studies that contained information on whether specific factors modified the O_3-associated health response. Details on this approach are provided in Sacks et al. (2011). Briefly, the focus was placed on studies that conducted stratified analyses (e.g., males vs. females), as these studies allow for a comparison between populations within the same study design. Experimental studies (toxicological and controlled human exposure) were also evaluated to inform coherence with the health effects observed in epidemiologic studies as well as provide an understanding of biological plausibility. Finally, we included those studies that examined RMFs that may result in differential air pollutant exposures and subsequently a greater risk of O_3-related health effects in a specific subset of the population, such as studies of outdoor workers.

Evaluation and characterization of scientific evidence

For each RMF the scientific evidence from each study evaluated was integrated across the scientific disciplines (i.e. epidemiologic, controlled human exposure, toxicological, and exposure sciences studies). It is through this integration that we applied the aspects described by Sir Austin Bradford Hill (Hill, 1965), which includes consistency within a discipline, coherence across disciplines, and biologically plausibility, to assess whether a specific factor resulted in a population or lifestage being at increased or decreased risk (Supplemental Material, Table S1).

When evaluating the collective evidence for a specific RMF, although the interpretation of individual studies is important, the focus is on the overall pattern of effects across studies. .For epidemiologic studies, effect measure modification was not necessarily deemed to be present if one comparison group had statistically significant findings while the other group did not. The evaluation of each study included in this overview focused on the examination of the magnitude, direction, and precision of the effect. Evidence of effect measure modification was noted when two comparison groups had different point estimates regardless of whether the point estimates were statistically significantly different as well as the degree of confidence interval overlap.

The weight-of-evidence approach used in this overview to assess whether specific factors modify the air pollutant (i.e., O_3)-health effect association is based upon the causal framework developed by the EPA to evaluate the causal nature of air pollution-related health or welfare effects and used in the ISAs (Supplemental Material, Table S2) (U.S. EPA 2013). Using this causal framework as a basis, we applied a weight-of-evidence approach, also utilized in the 2013 O_3 ISA (U.S. EPA 2013), to determine the level of confidence that a specific factor affects the risk of an air pollutant-related health effect. Table 1 characterizes the weight of evidence and considerations underlying each level of classification.

This overview focuses on those RMFs with sufficient evidence (i.e. adequate and suggestive) to draw a conclusion using the weight-of-evidence approach discussed above. We begin this overview by evaluating a RMF that has a strong biological component (genetic factors) and go through a range of potential RMFs, ending with one exclusively related to exposure (i.e., working outdoors). Details of each study are contained in Tables 2, 3, and the Supplemental Material, Tables S3 S4, and S5. An evaluation of all of RMFs resulted in some factors deemed to

have inadequate evidence and no factors demonstrated evidence of no effect. These factors are not discussed in this overview but are listed in Table 1.

Results and Discussion

Genetic factors

Specific genetic factors may affect the risk of health effects related to short- and long-term O_3 exposures, specifically polymorphisms in already identified candidate genes or in genes whose protein products are thought to be involved in the biological mechanism underlying the health effect of an air pollutant (Sacks et al. 2011). Previous reviews reported glutathione S-transferase Mu 1 (*GSTM1*) and tumor necrosis factor-α (*TNF*) to have a "potential role… in the innate susceptibility to O_3" (U.S. EPA 2006b). Table 2 provides a summary of recent effect measure modification findings for genetic variants examined in epidemiologic and controlled human exposure studies of respiratory effects. Due to small sample sizes, many controlled human exposure studies are limited in their ability to test genes with low frequency minor alleles. Among children with asthma, studies of children with the genetic variant of *GSTM1* null compared to *GSTM1* positive, have reported increased respiratory symptoms and decreased lung function (Romieu et al. 2006); Romieu et al. 2004b). Among healthy adults, studies have reported no effect of *GSTM1* variants on lung function and inconsistent results for inflammatory changes (Kim et al. 2011; Alexis et al. 2009) Studies of *GSTP1* have also reported a decrease in lung function and increase in respiratory symptoms (Alexeeff et al. 2008; Romieu et al. 2006; Romieu et al. 2004b). In controlled human exposure studies of *NQO1*, lung function among healthy adults was decreased for those with *NQO1* wildtype and *GSTM1* null gene variants (Bergamaschi et al. 2001). No difference was observed among asthmatics (Vagaggini et al.

2010). A study of *HMOX1* reported a potential decrease in lung function among adults (Alexeeff et al. 2008).

Toxicological studies have reported differences in effects after O_3 exposure among different inbred strains of mice, which indicates that genetic background contributes to differential risk (Chuang et al. 2009; Hamade and Tankersley 2009; Hamade et al. 2008; Tankersley et al. 2010). Inbred strains have been used in genetic linkage and genome-wide association studies to identify candidate genes that lead to increased risk (Cho and Kleeberger 2007), and additional studies have been conducted to validate these candidate genes and other related genes, primarily using mice with targeted gene deletions. Table 3 summarizes recent toxicological studies that examined the role of gene variants in the modification of the biological response to O_3 exposure. Overall, these studies demonstrate that genes related to innate immune signaling, in particular tumor necrosis factor receptors 1/2 and toll-like receptors 2/4 may modulate risk related to O_3 exposure, as well as associated genes including *Nfkb1*, *Jnk1*, *Cd44*, *Myd88, Iai*, *Hsp70*, *Mmp 9*, and *Nos2* (Cho et al. 2001; Cho et al. 2007; Williams et al. 2007; Hollingsworth et al. 2004; Kleeberger et al. 2000; Garantziotis et al. 2009; Bauer et al. 2011; Kleeberger et al. 2001; Fakhrzadeh et al. 2002; Kenyon et al. 2002; Yoon et al. 2007). There is also toxicological evidence indicating that genes involved in pro- and anti-inflammatory signaling and oxidative stress modulate O_3 response, including *Il10*, *Il13*, *Il6*, and *Cxcr2*, *Marco,* Csb, and *Nqo1* (Backus et al. 2010; Williams et al. 2008; Johnston et al. 2005a; Johnston et al. 2005b; Dahl et al. 2007; Kooter et al. 2008; Voynow et al. 2009). Taken together, this evidence demonstrates the complexity of the biological mechanisms underlying airway inflammation and AHR as well as genetic susceptibility, which has previously been described by Bauer and Kleeberger (2010).

Collectively, controlled human exposure and epidemiologic studies have reported evidence of O_3-related increases in respiratory symptoms or decreases in lung function with variants including *GSTM1*, *GSTP1*, *HMOX1* and *NQO1*. Toxicological studies of *NQO1* deficient mice reported that the mice were resistant to O_3-induced AHR and inflammation, providing biological plausibility for results of studies in humans. Additionally, studies of rodents have identified a number of other genes that may affect O_3-related health outcomes, including genes related to innate immune signaling, inflammation, and oxidative stress, which have not been investigated in human studies. Overall, there is "adequate evidence" indicating that certain genetic variants increase the risk of O_3-related health effects.

Lifestage

The 2010 Census reported that 27.0% of the U.S. population was under 20 years of age, with 13.1% under the age of 10 (Howden and Meyer 2011; SSDAN CensusScope 2010). Additionally, the number of older Americans (i.e., ≥ 65 years of age) is projected to increase from 12.4% to 19.7% of the U.S. population between 2000 to 2030 (U.S. Census Bureau 2010). Therefore, these lifestages represent a large population that may potentially be at increased risk of O_3-related health effects.

Both children and older adults are often considered to be intrinsically at increased risk of O_3-related health effects due to biological differences compared to the adult population. Respiratory systems of children are growing until 18-20 years of age (U.S. EPA 2006b). Young children also have greater lung regional extraction of O_3, which is thought to be due to smaller nasal and pulmonary region surface areas compared to the total airway surface area in adults (Sarangapani et al. 2003). Children have greater O_3 tissue doses in the lower airways due to higher ventilation rates per lung volume and a greater oral breathing contribution than adults (Becquemin et al.

1999; James et al. 1997; Bennett et al. 2008). Additionally, children often have higher exposure to O_3 than adults because children tend to spend more time outdoors (U.S. EPA 2013; U.S. EPA 2011a; Klepeis et al. 1996). Similar to children, older adults spend slightly more time outdoors than adults aged 18-64 years. However, older adults have somewhat lower ventilation rates than adults aged 31-60 years. The gradual decline in physiological processes that occur with aging may lead to increased risk of O_3-related health effects in older adults (U.S. EPA 2006a).

Controlled human exposure studies have demonstrated that children and adolescents appear, on average, to have nearly equivalent spirometric responses to O_3 exposure, but have greater responses than middle-aged and older adults (U.S. EPA 1996). Symptomatic responses (e.g., cough, shortness of breath, pain on deep inspiration) to O_3 exposure, however, increase with age until early adulthood and then gradually decrease with increasing age (U.S. EPA 1996; McDonnell et al. 1999). As a result, decreased symptomatic responses may put children and older adults at increased risk by withstanding continued O_3 exposure and thus not avoiding exposure. In addition, compared to younger age groups, older adults have a higher prevalence of preexisting diseases, with the exception of asthma, and this may also lead to increased risk of O_3-related health effects.

Epidemiologic studies have reported greater relative risks for O_3-related respiratory hospital admissions (HAs) and emergency department (ED) visits among children compared to adults (studies varied with adults defined as all ages over 15 or 18 years or 15 to 65 years of age) (Halonen et al. 2009; Middleton et al. 2008; Silverman and Ito 2010). However, some studies have reported positive associations among both children and adults with no evidence of effect measure modification by age (Ko et al. 2007; Paulu and Smith 2008; Mar and Koenig 2009).

The majority of multicity studies that present age stratified results conducted in the U.S. (Medina-Ramón and Schwartz 2008; Zanobetti and Schwartz 2008), Chile (Cakmak et al. 2007; Cakmak et al. 2011), and Italy (Stafoggia et al. 2010) as well as single city studies (e.g., (Kan et al. 2008)) found a trend of increased risk estimates for mortality due to short-term O_3 exposure in older adults (≥ 65 years old) compared to younger age groups. Exceptions include The Air Pollution and Health: A European and North American Approach (APHENA) (Katsouyanni et al. 2009), which only found increased percent change in mortality risk in the population ≥ 75 years in one study location (i.e., Canada), and a study conducted in Finland (Halonen et al. 2009), which found no evidence of an increased relative risk in the population ≥ 65 years old when compared to the population <65 years of age. Some of the studies that found evidence of an increased relative risk in older adults have shown inconsistent results when focusing on ages >85 years old, with the relative risk being higher in some cases (Stafoggia et al. 2010) and lower in others (Cakmak et al. 2007). A limited number of epidemiologic studies have examined potential differences in the relative risk by age in studies of respiratory-related HAs and ED visits (Halonen et al. 2009; Arbex et al. 2009) and studies of cardiovascular-related HAs (Halonen et al. 2009; Buadong et al. 2009) and reported generally inconsistent results. For the studies of cardiovascular–related HAs, results within the general population have been inconsistent and often null so it is plausible that no association would be observed regardless of age (U.S. EPA 2013).

Toxicological studies provide coherence for the potential increased relative risk of O_3-related health effects by age demonstrated in epidemiologic studies. Early life O_3 exposures of multiple species of laboratory animals, including infant monkeys and rodents, resulted in changes in conducting airways (e.g. Carey et al. 2007; Harkema et al. 1987; Plopper et al. 2007; Fanucchi et

al. 2006; López et al. 2008; Auten et al. 2009). Additionally, evidence indicates differences in inflammatory responses between neonatal and adult mice (Vancza et al. 2009; Bils 1970). Toxicological studies have also shown that oxidative damage and stress may be higher after O_3 exposure in young compared to adult rodents (Servais et al. 2005; Fortino et al. 2007). Additionally, a series of studies reported an association between O_3 exposure and bradycardia that was present among young but not older mice (Hamade and Tankersley 2009; Tankersley et al. 2010; Hamade et al. 2010). Physiologic changes specific to older adults that have been observed in toxicological studies include changes in heart structure (i.e., ventricular posterior wall thickness at end systole) (Tankersley et al. 2010), wound closure (Lim et al. 2006), and neurodegenerative diseases (as measured by higher lipid peroxidation in the hippocampus) (Rivas-Arancibia et al. 2000).

Generally, epidemiologic studies reported larger associations for respiratory HA and ED visits for children than adults. However, the interpretation of these studies is limited by the lack of consistency in comparison age groups and outcomes examined. For older adults, epidemiologic studies are primarily limited to those examining short-term O_3 exposure and mortality, but provide evidence of consistent positive associations in older adults when compared to younger age groups. These results are supported by toxicological studies that demonstrate effects in younger (i.e., morphological changes to lung structure) and older animals (i.e., physiologic changes). Also, children and older adults may experience increased exposure due to time spent outdoors, differences in lung regional extraction of O_3 (children), ventilation rates, and reduction in physiologic response to O_3 exposures with increasing age. Overall, there is "adequate evidence" indicating that certain lifestages (children and older adults), are at increased risk of O_3-related health effects.

Sex

Epidemiologic studies that examined potential differences by sex in associations between O_3 exposure and respiratory HAs have not consistently found larger relative risk estimates in one group compared to another (Cakmak et al. 2006a; Middleton et al. 2008). For example, there is evidence for higher relative risk estimates in females compared to males in studies of chronic obstructive pulmonary disease HAs and ED visits (Arbex et al. 2009; Wong et al. 2009). However, in studies of asthma ED visits, differences between males and females were observed by age; larger relative risk estimates were reported for males age 2-14 and females age 15-34, with no evidence of any sex differences in age 35-64 (Paulu and Smith 2008). These results are consistent with Thaller et al. (2008), which found evidence of decreased lung function in females compared to males aged 16-27 years old. Additionally, Lin et al. (2005) found no evidence for differences in males and females when examining respiratory infection HAs in individuals <15 years of age.

A number of epidemiologic studies that examined cardiovascular-related HAs and ED visits reported no effect modification by sex with some studies reporting null associations for both males and females (Middleton et al. 2008; Wong et al. 2009; Villeneuve et al. 2006;Henrotin et al. 2007) and one study reporting a positive associations for both sexes (Cakmak et al. 2006b). However, as mentioned previously, the lack of evidence for effect measure modification by sex may be indicative of the lack of association with cardiovascular morbidity, not the lack of an effect by sex (U.S. EPA 2013).

A few epidemiologic studies have examined the association between short-term O_3 exposure and mortality stratified by sex and, in contrast with studies of other endpoints, the evidence was more consistent in reporting elevated relative risks among females. These studies, conducted in the

U.S. (Medina-Ramón and Schwartz 2008), Italy (Stafoggia et al. 2010), and Asia (Kan et al. 2008), reported larger effect estimates in females compared to males with some evidence of the relative risk of mortality among females being larger specifically among those ≥ 60 years old (Medina-Ramón and Schwartz 2008). However, another study did not find any difference in the relative risk of O_3-related mortality among men and women (Cakmak et al. 2011).

Experimental studies describe biologically plausible mechanisms that may explain differential risk in O_3-related health effects between males and females, however some uncertainty remains. Several controlled human exposure studies have suggested that physiological differences between sexes may predispose females to greater effects from O_3. Specifically, in females, lower plasma and nasal lavage fluid (NLF) levels of uric acid, the initial defense mechanism of O_3 neutralization, may result in females being at increased risk of O_3-related health effects (Housley et al. 1996). Consequently, reduced absorption of O_3 in the upper airways of females may promote its deeper penetration. A toxicological study, Vancza et al. (2009) found small differences in effects by sex in adult mice with respect to pulmonary inflammation and injury after O_3 exposure, with adult females generally more at risk. However, these differences were strain-dependent, with some strains exhibiting greater risk in males. The most obvious sex difference in this study was in lactating females, which incurred the greatest lung injury or inflammation among several of the strains. However, not all studies have found differences in the physiologic response to O_3 exposure; FEV1 responses in young healthy females appeared comparable to the response of young males (Hazucha et al. 2003). When evaluating the potential for sex differences in O_3 absorption, Bush et al. (1996) reported that the absorption distribution of O_3 was independent of sex when absorption was normalized to anatomical dead space.

Epidemiologic studies of O_3 exposures and mortality found evidence of elevated relative risks in females; whereas studies of respiratory morbidity found inconsistent results with some evidence of differences in relative risk by sex depending on age. Although experimental studies provide potential biological plausibility for potential differences by sex, these studies have not consistently demonstrated a clear difference in O_3-related effects by sex and could potentially be explained by differences in anatomical dead space volume. As a result, across disciplines there is "suggestive evidence" for differences in risk by sex.

Asthma

Within the U.S. in 2008, approximately 7.3% of adults and 9.5% of children reported currently having asthma (Pleis et al. 2009; Bloom et al. 2009). As a result, disproportionate impacts of O_3 exposure on the population of individuals with asthma could result in a significant public health impact.

Epidemiologic studies have not consistently demonstrated decreased lung function in asthmatics compared to non-asthmatics in response to short-term O_3 exposure (Thaller et al. 2008). However, there is some evidence of increased relative risks for wheeze and cough among asthmatics but not non-asthmatics, although this may have been the result of a small non-asthmatic population in this study (Escamilla-Nuñez et al. 2008). Greater short-term,O_3-associated, decreases in lung function have been observed in older individuals with airway hyperresponsiveness (AHR), a sign of asthma, compared to those without AHR (Alexeeff et al. 2007). Additionally, studies have demonstrated that short-term O_3 exposure is associated with airway inflammation in children regardless of their asthmatic status (Barraza-Villarreal et al. 2008; Berhane et al. 2011). The inconsistency in results across these epidemiologic studies could be due to the studies not accounting for behavioral responses. A recent study has found

that not taking into account individual behavioral adaptations to forecasted air pollution levels (such as avoidance and reduced time outdoors) can underestimate observed associations between short-term O_3 exposures and respiratory effects (Neidell and Kinney 2010).

Similar to the evidence from epidemiologic studies, controlled human exposure studies that compare asthmatics to healthy controls demonstrate that subjects with asthma appear to be at least as sensitive to the acute effects of O_3 in terms of FEV_1 and inflammatory responses as healthy non-asthmatic subjects. Multiple studies have demonstrated that asthmatics experience greater O_3-related FEV_1 decrements than healthy subjects (Horstman et al. 1995; Kreit et al. 1989; Alexis et al. 2000; Jorres et al. 1996). However, another study reported individuals with asthma to have smaller O_3-related FEV_1 decrements than healthy subjects, but the asthmatics in the study tended to be older than the healthy subjects, which could partially explain their smaller response since FEV_1 responses to O_3 exposure have been shown to diminish with age (Mudway et al. 2001). Controlled human exposure studies have also reported subclinical changes in individuals with asthma, compared to similarly exposed healthy individuals, including increased bronchoalveolar lavage fluid (BALF) neutrophils, higher levels of cytokines and hyaluronan in lavage fluid or sputum, and greater expression of macrophage cell-surface markers, which provide biological plausibility for increased O_3-related health effects in observed in asthmatics (Basha et al. 1994; Peden et al. 1997; Scannell et al. 1996; Hernandez et al. 2010; Bosson et al. 2003).

Toxicological studies are coherent with other studies showing greater O_3 effects among those with asthma or AHR. Using an asthmatic phenotype modeled by allergic sensitization of the respiratory tract, effects of O_3 on pulmonary function were found to be augmented by allergic sensitization in infant rhesus monkeys (Fanucchi et al. 2006; Joad et al. 2006; Schelegle et al.

2003), mice (Funabashi et al. 2004), and rats (Wagner et al. 2007). In addition, in a bleomycin induced pulmonary fibrosis model, exposure to O_3 increased pulmonary inflammation and fibrosis, along with the frequency of bronchopneumonia in rats (Oyarzún et al. 2005). Thus, short-term O_3 exposure may also enhance damage in a previously injured lung.

Epidemiologic studies demonstrate that asthmatics are at least as sensitive as healthy individuals to O_3-related health effects. Controlled human exposure studies have shown increased FEV1 decrements and inflammatory responses in asthmatics compared to healthy individuals. Finally, controlled human exposure and toxicological studies using animal models of asthma provide biological plausibility for the effects observed in some epidemiologic studies. Overall, there is "adequate evidence" that people with asthma are at increased risk of O_3-related health effects.

Obesity

Obesity, defined as a body mass index (BMI) of 30 kg/m^2 or greater, is an issue of increasing importance in the U.S., with self-reported rates of obesity of 26.7% in 2009, up from 19.8% in 2000 (Sherry et al. 2010). Recent studies of air pollution have begun to examine whether obesity is a risk factor for air pollution-related health effects. An epidemiologic study reported decreased lung function with increased short-term O_3 exposure for both obese and non-obese subjects; however, the magnitude of the reduction in lung function was greater for those subjects who were obese (Alexeeff et al. 2007). Further decrements in lung function were noted for obese individuals who also had AHR.

Controlled human exposure studies have detected differential effects of O_3 exposure on lung function for individuals with varying BMIs. In a retrospective analysis of data from healthy, nonsmoking, white males between the ages of 18-35 years, increased BMI was associated with

enhanced FEV_1 responses (McDonnell et al. 2010). In a similar analysis, greater O_3-related FEV_1 decrements were observed with increasing BMI in a group of healthy, nonsmoking, women (BMI range 15.7 to 33.4 kg/m^2), but not among healthy, nonsmoking men (BMI range 19.1 to 32.9 kg/m^2) indicating that sex may also play a role in any O_3 effects attributed to obesity (Bennett et al. (2007)).

Animal toxicological studies have also reported enhanced pulmonary inflammation and injury with acute O_3 exposure in genetic and diet-induced obese mice, providing biological plausibility and coherence with the effects observed in epidemiologic and controlled human exposure studies (reviewed in Shore 2007, Johnston et al. 2008). However, a recent study found that obese mice are resistant to O_3-related pulmonary injury and inflammation and reduced lung compliance following longer exposures at lower concentrations, regardless of whether obesity was genetic- or diet-induced (Shore et al. 2009).

Epidemiologic and controlled human exposure studies report evidence for increased O_3-related respiratory health effects among obese individuals. Toxicological studies are generally coherent with evidence in epidemiologic and controlled human exposure studies. Some, but not all, studies support the possibility of increased risk of O_3-related pulmonary effects among obese individuals. Overall, there is "suggestive evidence" that obese individuals are at increased risk of O_3-related health effects.

Diet

Diet, which is strongly correlated with other factors, such as obesity and socioeconomic status (SES), may modify the association between O_3 exposure and health effects. Ozone mediates some of its toxic effects through oxidative stress (U.S. EPA 2013); therefore, the antioxidant

status of an individual is an important factor that may affect the risk of O_3-related health effects. As a result, a number of studies have examined dietary factors, specifically, supplementation with antioxidant vitamins (e.g., C and E), to identify whether these factors inhibit O_3-mediated damage.

In epidemiologic studies, increases in fruit/vegetable intake and Mediterranean dietary patterns, which have been noted for their high vitamins C and E and omega-3 fatty acid content, were found to protect against O_3-related decreases in lung function among children (Romieu et al. 2009). Similarly, the protective effect of dietary supplementation in asthmatic children was demonstrated by an association between short-term O_3 exposure and nasal airway inflammation among a placebo group, but not among a group supplemented with vitamins C and E (Sienra-Monge et al. 2004).

Results from epidemiologic studies are consistent with those observed in controlled human exposure studies that provide evidence of protective effects of α-tocopherol (a form of vitamin E) and ascorbate (vitamin C) on spirometric measures of lung function after O_3 exposure, but not on the intensity of subjective symptoms and inflammatory response including cell recruitment, activation and release of mediators (Samet et al. 2001; Trenga et al. 2001). Dietary antioxidants have also afforded protection to asthmatics by attenuating postexposure bronchial hyperresponsiveness (Trenga et al. 2001).

Toxicological studies also provide evidence of protective effects from vitamin supplementation which is consistent with evidence from epidemiologic and controlled human exposure studies. Studies in rats show that γ-tocopherol treatment inhibits O_3-related inflammation and mucus production and reduces O_3-exacerbated nasal allergy responses (Wagner et al. 2007; Wagner et

al. 2009). Similarly, vitamins C and E supplementation led to attenuation of inflammation, oxidative stress, and airway hyperresponsiveness in guinea pigs exposed subchronically to O_3 (Chhabra et al. 2010). However, in another study, guinea pigs deficient in vitamin C displayed only minimal differences in injury and inflammation after exposure to O_3 compared to vitamin C sufficient animals (Kodavanti et al. 1995). Additional studies have provided evidence that β-carotene and vitamin A supplementation protected against the effects of O_3 exposure (Valacchi et al. 2009; Paquette et al. 1996).

Consistent evidence across disciplines demonstrates that individuals with reduced intake of vitamins E and C are at increased risk for O_3-related health effects. The evidence from epidemiologic studies is supported by controlled human exposure and toxicological studies, and collectively provides "adequate evidence" that individuals with an insufficient diet are at increased risk of O_3-related health effects.

Socioeconomic status (SES)

SES is often represented by personal or neighborhood SES, which is comprised of a variety of components such as educational attainment, household income, and health insurance status. SES is typically indicative of such things as access to healthcare, quality of housing, and pollution gradient to which people are exposed.

Multiple epidemiologic studies report that individuals of low SES have an increased relative risk of respiratory effects (e.g., HAs and ED visits) due to O_3 exposures. This includes studies that examined SES using neighborhood-level educational attainment in Canada (Cakmak et al. 2006a) and regional insurance rates in Korea (Lee et al. 2006). However, some studies, specifically in Canada, have found no evidence of modification of the relative risk using

measures of neighborhood-level income (Cakmak et al. 2006a; Burra et al. 2009). SES was also examined in a study of short-term O_3 exposures and cardiac disease ED visits in Canada where neighborhood-level education or income was divided into quartiles (Cakmak et al. 2006b). The authors did not observe effect measure modification of cardiac disease ED visits by any level of neighborhood education or income, which may not necessarily inform SES differences overall due to the limited evidence for O_3-induced cardiovascular HAs and ED visits in the general population, as mentioned previously.

Several large scale epidemiologic studies (i.e., NMMAPS and APHENA) reported increased relative risk of O_3-related mortality among groups with lower SES based on neighborhood-level unemployment in the United States (Bell and Dominici 2008; Katsouyanni et al. 2009). Increases in O_3-related mortality have also been observed in studies using individual-level education, individual-level occupation, and neighborhood-level income as measures of SES (Cakmak et al. 2011). Other studies conducted in China and Italy reported inconsistent or null findings using individual-level educational attainment (Kan et al. 2008), a neighborhood-level deprivation index (Wong et al. 2008), and neighborhood-level income (Stafoggia et al. 2010). The influence of SES on mortality has also been examined in studies of infant mortality in Mexico. These studies found no association between O_3 concentrations and infant mortality regardless of SES, measured using neighborhood-level indicators, such as income or availability of public services (Romieu et al. 2004a; Carbajal-Arroyo et al. 2011); however, in a study by Carbajal-Arroyo et al. (2011) there was evidence of a positive association for respiratory-related infant mortality in only the low SES group.

Morello-Frosch et al. (2010) reported greater decreases in birth weight associated with full pregnancy O_3 concentration for those with higher neighborhood poverty rates. However, a study

conducted in Australia using a neighborhood-level SES index comprised of multiple factors, such as income and unemployment, demonstrated no modification of the association between O_3 exposure during days 31-60 of gestation and abdominal circumference during gestation despite some evidence of an inverse association in the highest SES quartile (Hansen et al. 2008).

A single controlled human exposure study examined O_3 effects on lung function and potential modification of response among three SES categories (based on father's educational attainment), although the study was not originally designed to investigate SES (Seal et al. 1996). Individuals in the middle SES category showed greater concentration-dependent decline in percent-predicted FEV_1 than the low and high SES groups. However, it is unclear why differences were greatest in the middle SES group in this study.

Most studies have reported that individuals with low SES or living in neighborhoods with low SES have an increased relative risk of O_3-related respiratory HA and ED visits. Inconsistent results have been observed in the few studies examining effect measure modification of the O_3 association with mortality and reproductive outcomes. A controlled human exposure study, although not designed to examine differences by SES, does not support evidence of increased risk of O_3-related health effects among individuals with lower SES. Overall, there is "suggestive evidence" that individuals of low SES are at increased risk of experiencing O_3-related health effects.

Outdoor workers

Multiple epidemiologic studies have found that individuals who participate in outdoor activities or work outside to be a population at increased risk of air pollution-related health effects due to increased exposure, which has been affirmed by studies that have reported consistent

associations between O_3 exposure and respiratory health outcomes in these groups (U.S. EPA 2006b). Outdoor workers are exposed to ambient O_3 concentrations for a greater period of time than individuals who spend their days indoors. Additionally, an increase in dose to the lower airways in this population is expected due to both increases in the amount of air breathed (i.e., minute ventilation) and a shift from nasal to oronasal breathing that traditionally occurs during outdoor exercise (Hu et al. 1994; Nodelman and Ultman 1999). However, the health effects in this population seem to be limited to respiratory-related effects as evidenced by an epidemiologic study exploring effect measure modification of O_3 exposure by workplace (i.e. indoor/outdoor) on DNA damage, which found inconsistent results (Tovalin et al. 2006).

There is strong evidence demonstrating increased exposure, dose, and ultimately risk of O_3-related respiratory effects in outdoor workers. Overall, there is "adequate evidence" that outdoor workers are at increased risk of O_3-related health effects.

Limitations

We recognize in some cases, it is difficult to clearly determine if a factor leads to increased or decreased risk of a population experiencing O_3-related health effects. Not only is this due to inconsistencies within a discipline, the lack of coherence across disciplines, or the lack of biological plausibility, but also intersubject variability and the possible attenuation of O_3-related effects. Controlled human exposure studies have clearly demonstrated intersubject variability in respiratory-related responses to O_3 exposure among healthy adults (Holz et al. 2005; McDonnell 1996; Que et al. 2011). These responses tend to be reproducible within a given individual over a period of several months indicating differences in the intrinsic responsiveness (Hazucha et al. 2003; Holz et al. 2005; McDonnell et al. 1985; Holz et al. 1999). In addition, pre-exposure to O_3

leads to an attenuation of lung function and symptomatic responses to O_3 on subsequent days (Foxcroft and Adams 1986).

Inconsistency in the categorization and/or measurement of a factor across studies makes drawing conclusions regarding potential RMFs difficult. For example, numerous metrics are used to characterize SES. Additionally, when considering epidemiologic studies conducted in other countries, it should be noted that it is possible those populations may differ in SES or other demographic indicators (e.g., overall health status), thus limiting generalizability to a U.S. population.

Additionally, there is the possibility of publication bias. Stratified analyses that have interesting effect measure modification results may be presented in publications, whereas studies that find no evidence of effect measure modification may not. It is not possible to measure the influence of publication bias on our overall conclusions; therefore, some of the evidence may be more varied than presented.

Finally, we recognize that additional studies that could inform the conclusions we reached in our evaluation of the scientific evidence could have been missed in our literature search. We focused on searches using Web of Science and PubMed, and did not utilize other databases, such as, EMBASE. Additionally, our systematic literature search was limited to recent years, however informative studies included in past assessments were also included. The literature summarized in this overview was drawn from the 2013 O_3 ISA (U.S. EPA 2013), which was reviewed by scientific experts and had an associated call for papers/public comment. Therefore, we are confident that all relevant papers were captured.

Conclusions

The integration of evidence across scientific disciplines, which allows for an evaluation of the consistency of effects within and the coherence of effects across disciplines, as well as an evaluation of biological plausibility provide a scientific basis for drawing conclusions regarding populations that are potentially at increased or decreased risk of an air pollutant-related health effect. Based on our evaluation of the scientific evidence, we concluded that there is "adequate" evidence for increased risk of O_3-related health effects in population groups with certain genotypes, preexisting asthma, certain lifestages (i.e., younger and older age groups), populations with reduced intake of certain nutrients, and outdoor workers (Table 1). Other factors (i.e., sex, SES, and obesity) were characterized by "suggestive" evidence for increased risk of O_3-related health effects.

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Table 1. Classification of evidence for potential response-modifying factors.

Level of evidence	Criteria	Potential response- modifying factors
Adequate evidence	There is substantial, consistent evidence within a discipline to conclude that a factor results in a population or lifestage being at increased or decreased risk of air pollutant-related health effect(s) relative to some reference population or lifestage. Where applicable this includes coherence across disciplines. Evidence includes multiple high-quality studies.	Genetic factors, asthma, children, older adults, diet, outdoor workers
Suggestive evidence	The collective evidence suggests that a factor results in a population or lifestage being at increased or decreased risk of an air pollutant-related health effect relative to some reference population or lifestage, but the evidence is limited due to some inconsistency within a discipline or, where applicable, a lack of coherence across disciplines.	Sex, socioeconomic status, obesity
Inadequate evidence	The collective evidence is inadequate to determine if a factor results in a population or lifestage being at increased or decreased risk of an air pollutant-related health effect relative to some reference population or lifestage. The available studies are of insufficient quantity, quality, consistency and/or statistical power to permit a conclusion to be drawn.	Influenza/Infection, chronic obstructive pulmonary disease, cardiovascular disease, diabetes, hyperthyroidism, race/ethnicity, smoking, air conditioning use
Evidence of no effect	There is substantial, consistent evidence within a discipline to conclude that a factor does not result in a population or lifestage being at increased or decreased risk of air pollutant-related health effect(s) relative to some reference population or lifestage. Where applicable this includes coherence across disciplines. Evidence includes multiple high-quality studies.	None identified

Table 2. Summaries of results from epidemiologic and controlled human exposures studies of modification by genetic variants.

Gene variant	Comparison group	Health outcome /population	Effect modification of association for the gene variant	Source
GSTM1 null	*GSTM1* positive	Lung function among healthy adults with intermittent moderate exercise	Exposed 0.4 ppm, 2h; Results: At 24 hrs post-exposure, both groups had decreased FEV1 and FVC, with no reported difference between the groups	Alexis et al. (2009)
GSTM1 null	*GSTM1* positive	Inflammatory changes among healthy adults with intermittent moderate exercise	Exposed 0.4 ppm, 2h; Results: At 24 hrs post-exposure, GSTM1 null had increased polymorphonuclear neutrophils numbers, increased oxidative burst, increased phagocytic function, increased expression of CD14 on airway polymorphonuclear neutrophils, increased expression of HLA-DR on airway dendritic cells, increased expression of HLA-DR on macrophages, increased IL-1β and IL-8 compared to GSTM1 positive	Alexis et al. (2009)
GSTM1 null	*GSTM1* positive	Lung function among healthy adults with intermittent moderate exercise	Exposed 0.06 ppm, 6.6h; Results: Both groups had decreased FEV1 and FVC, with no reported difference between the groups; no difference was observed for symptom scores between the two groups	Kim et al. (2011)
GSTM1 null	*GSTM1* positive	Inflammatory responses among healthy adults with intermittent moderate exercise	Exposed 0.06 ppm, 6.6h; Results: Both groups had increased percentage of polymorphonuclear neutrophils	Kim et al. (2011)
GSTM1 null	*GSTM1* positive	Respiratory symptoms among asthmatic children	Air concentrations mean 8-h max 69 ppb (SD 31 ppb); Results: Both groups had decreased FEV1 and FVC, with no reported difference between the groups; no difference was observed for symptom scores between the two groups	Romieu et al. (2006)
GSTM1 null	*GSTM1* positive	Lung function among asthmatic children	Air concentrations mean 1-h max 102 ppb (SD 47 ppb); Results: GSTM1 null had decreased FEF_{25-75} compared to GSTM1 positive in the placebo group, but no difference between genotypes for the group with antioxidant supplementation	Romieu et al. (2004b)
GSTP1 Ile/Val or Val/Val	*GSTP1* Ile/Ile	Lung function among adults	Air concentrations mean 2-d 24.4 ppb (SD 11 ppb); Results: Decreased FEV1 for HMOX1 S/L or L/L compared to S/S; possibly decreased for FVC as well but confidence interval overlap present	Alexeeff et al. (2008)

Gene variant	Comparison group	Health outcome /population	Effect modification of association for the gene variant	Source
GSTP1 Val/Val	*GSTP1* Ile/Ile or Ile/Val	Respiratory symptoms among asthmatic children	Air concentrations mean 8-h max 69 ppb (SD 31 ppb); Results: GSTP1 Val/Val had increased difficulty breathing, increased bronchodilator use, and increased cough compared to GSTP1 Ile/Ile or Ile/Val	Romieu et al. (2006)
GSTP1 Ile/Ile or Ile/Val	*GSTP1* Val/Val	Lung function among asthmatic children	Air concentrations mean 8-h max 69 ppb (SD 31 ppb); Results: Decreased FEV1 and FVC possible for GSTP1 Ile/Ile or Ile/Val compared to Val/Val but the null was included in all estimates	Romieu et al. (2006)
HMOX1 S/L or L/L	*HMOX1* S/S	Lung function among adults	Air concentrations mean 2-d 24.4 ppb (SD 11 ppb); Results: Decreased FEV1 and FVC possible for HMOX1 S/L or L/L compared to S/S but confidence interval overlap present	Alexeeff et al. (2008)
NQO1 wildtype and *GSTM1* null	Other combinations[a]	Lung function among healthy adults with exercise	Air concentrations median 2-h 78 ppb; Results: Decreased FEV1 for NQO1 wildtype and GSTM1 null compared to other combinations, similar FVC changes observed in both groups	Bergamaschi et al. (2001)
NQO1 wildtype and *GSTM1* null	Other combinations[a]	Lung function among mild-to-moderate asthmatics with moderate exercise	Exposed 0.3 ppm, 2h; Results: No difference in FEV1 between the groups	Vagaggini et al. (2010)
NQO1 wildtype and *GSTM1* null	Other combinations[a]	Inflammatory responses among mild-to-moderate asthmatics with moderate exercise	Exposed 0.3 ppm, 2h; Results: Quantitative results not provided but stated “no difference in the inflammatory response for ozone exposure was found between…” the two groups; tests conducted by the researchers included macrophages, lymphocytes, neutrophils, eosinophils, IL-8, etc.	Vagaggini et al. (2010)

[a]E.g. NQO1 defective and GSTM1 positive/null.

Table 3. Summaries of results from animal toxicology studies of modification by genetic variants[a].

Gene variant	Source	Exposure	Health outcome
Cd44	Garantziotis et al. (2009)	2.0ppm, 3hr	Decreased AHR.
Csb	Kooter et al. (2008)	1.0ppm, 3hr	Decreased BALF Tnf-α. No genotype effect on neutrophilia or epithelial damage.
Cxcr2	Johnston et al. (2005a)	1.0ppm, 3hr	Decreased neutrophilia, epithelial injury, and AHR. No genotype effect on hyperpermeability.
Hsp70	Bauer et al. (2011)	0.3ppm, 48hr	Decreased hyperpermeability and inflammation.
Iai	Garantziotis et al. (2009)	2.0ppm, 3hr	Decreased AHR.
Il6	Johnston et al. (2005b)	0.3ppm, 3 or 72hr	Decreased soluble TNFR1.
Il6	Johnston et al. (2005b)	0.3ppm, 72hr	Decreased hyperpermeability, BALF neutrophils, and soluble TNFR1 and TNFR2. No genotype effect on AHR.
Il6	Johnston et al. (2005b)	2.0ppm, 3hr	Reduced BALF neutrophils and soluble TNFR2 and MIP-2.
Il10	Backus et al. (2010)	0.3ppm, 24, 48, and 72hr	Increased inflammation No genotype effect on hyperpermeability.
Il13	Williams et al. (2008)	3.0ppm, 3hr	Decreased neutrophilia, BALF cells, and AHR.
Jnk	Cho et al. (2007)	0.3ppm, 48hr	Decreased hyperpermeability, neutrophilia, and epithelial damage.
Marco	Dahl et al. (2007)	0.3ppm, 48hr	Increased inflammation and hyperpermeability.
Mmp7	Yoon et al. (2007)	0.3ppm, 48hr	No genotype effect on hyperpermeability or inflammation.
Mmp9	Yoon et al. (2007)	0.3ppm, 48hr	Increased hyperpermeability, neutrophilia, and lung damage.
Myd88	Williams et al. (2007)	3.0ppm, 3hr	Decreased inflammation, hyperpermeability, and AHR
Nfkb1	Cho et al. (2007)	0.3ppm, 48hr	Decreased hyperpermeability, neutrophilia, and epithelial damage.
Nos2	Kleeberger et al. (2001)	0.3ppm, 72hr	Decreased hyperpermeability. No genotype effect on neutrophilia or epithelial damage.
Nos2	Fakhrzadeh et al. (2002)	0.8ppm, 3hr	Decreased hyperpermeability and BALF cells. Decreased peroxynitrite, COX1, and COX2 production and increased superoxide anion and PGE_2 production in alveolar macrophages.
Nos2	Kenyon et al. (2002)	1.0ppm, 8hr/night, 3 nights	Increased hyperpermeability and inflammation.
Nqo1	Voynow et al. (2009)	1.0ppm, 3hr	Decreased inflammation and AHR.
Tlr2	Williams et al. (2007)	3.0ppm, 3hr	Decreased inflammation and AHR. No genotype effect on hyperpermeability.
Tlr4	Kleeberger et al. (2000)	0.3ppm, 72hr	Decreased hyperpermeability
Tlr4	Hollingsworth et al. (2004)	0.3ppm, 72hr	No effect on AHR, hyperpermeability, or neutrophilia
Tlr4	Hollingsworth et al. (2004)	2.0ppm, 3hr	Decreased AHR. No genotype effect on hyperpermeability or neutrophilia
Tlr4	Williams et al. (2007)	3.0ppm, 3hr	Decreased AHR and neutrophilia. No genotype effect on hyperpermeability.
Tnfr1/Tnfr2	Cho et al. (2007), Cho et al. (2001)	0.3ppm, 48hr	Decreased inflammation and epithelial damage. No genotype effect on hyperpermeability.
Tnfr1/Tnfr2	Cho et al. (2007), Cho et al. (2001)	2.0ppm, 3hr	Decreased AHR. No genotype effect on neutrophilia, hyperpermeability, or epithelial damage.

[a]The table includes animal toxicology studies where responses are assessed after gene deletion with the exception of Kleeberger et al. 2000 that compared *C3H/HeJ* (*Tlr4* mutant) to *C3H/HeOuJ* (*Tlr4* normal) mice.